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Combined Effect of Sulfur Dioxide and Ozone on Bean and Tobacco Plants

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16. ABSTRACT

Plants of two cultivars of Phaseolus vulgaris L. and one cultivar of Nicotiana tabacum L. were exposed to a replicated series of concentrations of sulfur dioxide (SO_2), ozone (O_3), and combinations of these two air pollutants for single four-hour periods. Experiments were performed in controlled-environment chambers using concentrations of pollutants above and below Federal ambient air quality standards in order to determine whether interactive effects result from exposure to concentrations of pollutants occurring in some agricultural areas of the United States. Dose-response curves for foliar symptoms were found to approximate logarithmic functions of pollutant concentrations when the frequency of leaf injury was converted into probit units. In bean cultivars, the toxicity of SO_2 or O_3 was either attenuated or not affected when the two pollutants were combined depending on their concentrations. In tobacco, the toxicity of SO_2 or O_3 was either amplified or not affected in the presence of the two pollutants. Concentrations of SO_2 or O_3 that did not exceed the respective U.S. ambient air quality standards significantly altered the incidence of injury caused by the other pollutant. These results demonstrate that interactive effects between SO_2 and O_3 can occur but the magnitude and direction of these effects depend on plant species and concentrations of pollutants.

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THE COMBINED EFFECT OF SULFUR DIOXIDE AND OZONE ON BEAN AND TOBACCO PLANTS

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JACOBSON J. S. AND COLAVITO, L. J. *The combined effect of sulfur dioxide and ozone on bean and tobacco plants.* ENVIRONMENTAL AND EXPERIMENTAL BOTANY **16,** 277–285, 1976.—Plants of two cultivars of *Phaseolus vulgaris* L. and one cultivar of *Nicotiana tabacum* L. were exposed to a replicated series of concentrations of sulfur dioxide (SO_2), ozone (O_3), and combinations of these two air pollutants for single four-hour periods. Experiments were performed in controlled-environment chambers using concentrations of pollutants above and below Federal ambient air quality standards in order to determine whether interactive effects result from exposure to concentrations of pollutants occurring in some agricultural areas of the United States. Dose–response curves for foliar symptoms were found to approximate logarithmic functions of pollutant concentrations when the frequency of leaf injury was converted into probit units. In bean cultivars, the toxicity of SO_2 or O_3 was either attenuated or not affected when the two pollutants were combined depending on their concentrations. In tobacco, the toxicity of SO_2 or O_3 was either amplified or not affected in the presence of the two pollutants. Concentrations of SO_2 or O_3 that did not exceed the respective U.S. ambient air quality standards significantly altered the incidence of injury caused by the other pollutant. These results demonstrate that interactive effects between SO_2 and O_3 can occur but the magnitude and direction of these effects depend on plant species and concentrations of pollutants.

INTRODUCTION

CURRENT AIR quality regulations designed to reduce the risk of injury to vegetation from air pollutants are based on the response of plants to individual gases. However, pollutants frequently occur in combinations and studies performed with individual gases may not explain plant response to mixtures of pollutants. Thus, the current United States secondary air quality standard for ozone (O_3), 0·08 μl/l for a one-hour averaging period,[7]† may not adequately protect certain plant species in the presence of sulfur dioxide (SO_2), and the standard for SO_2, 0·5 μl/l for a three-hour averaging period,[7]† may be inappropriate in the presence of O_3. The standard for O_3 is exceeded frequently at many suburban and rural locations in the United States during the growing season due to the formation of O_3 by photochemical reactions involving nitrogen oxides and hydrocarbon compounds. Elevated SO_2 concentrations also occur in many areas as a result of combustion of fossil fuels. Both O_3 and SO_2 cause injury to vegetation during the growing season and experimental results have indicated that injury to deciduous vegetation may be amplified,[2, 11, 17, 20, 21, 26–28] attenuated,[6, 24–26] or not affected[25–28] by the combined presence of SO_2 and O_3. Dose–response relationships for mixtures of SO_2 and O_3 have been fully evaluated only for a single plant species.[17]

A theoretical basis for understanding the joint

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† 0·08 μl/l O_3 = 0·08 ppm v/v = 0·16 mg/m^3; 0·5 μl/l SO_2 = 0·5 ppm v/v = 1·3 mg/m^3 at 20° C and 760 mm Hg.

effects of toxic agents was established by BLISS[4] and later extended to aerosols,[16] fungicides,[3,29] the mammalian toxicity of air contaminants,[5] and insecticides.[23] The terms synergism and antagonism have been used in different ways in publications on the combined effects of toxic agents, consequently, their meaning has become ambiguous. In addition, quantitative relationships between pollutant dose and plant response have seldom been determined over a range of concentrations for mixtures of pollutants, so it has not been possible to draw firm conclusions concerning synergism or antagonism.

The specific objectives of this study were to determine whether the foliar response of plants to SO_2 is altered by a concentration of O_3 below the ambient air quality standard and whether the foliar response to O_3 is altered by a concentration of SO_2 below the standard. The experiments were performed over a range of SO_2 and O_3 concentrations under controlled environmental conditions for two cultivars of bean and one cultivar of tobacco in order to provide quantitative measures of dose–response relationships. Bean and tobacco are known to be susceptible to O_3 and the Bel-W 3 cultivar of tobacco is a commonly-used, highly susceptible plant indicator for O_3.[15]

MATERIALS AND METHODS

The procedures used for reporting the results of these experiments were based on a published guideline for studies carried out in growth chambers.[1]

Plants and their culture

Nicotiana tabacum L. "Bel-W 3" and *Phaseolus vulgaris* L. "Red Kidney" were grown in greenhouses that received air filtered through activated charcoal, and *P. vulgaris* "Tempo" was grown in controlled-environment chambers. One tobacco plant or 2 bean plants were grown separately in 4-inch pots in soil and a commercial peat–vermiculite medium (Jiffy-Mix), respectively. Between 20–80 individual bean plants were exposed to pollutants when unifoliate leaves were fully expanded and the first trifoliate leaves were beginning to expand (about 9 days for Tempo and 14 days for Red Kidney). Preliminary tests indicated that unifoliate leaves were most susceptible to SO_2 and O_3 at this developmental stage. Four to 15 tobacco plants were exposed to pollutants when 6 to 14 leaves were present, 8 to 12 weeks after seedling.

Treatment

Tempo bean plants were grown in chambers at $24 \pm 0{\cdot}5°C$, $70 \pm 5\%$ r.h. and all plants were exposed to pollutants for a period of 4 hr in the middle of the 16-hr photoperiod. Light intensities at plant height of 1800, 990, and 510 $\mu W^2 \times cm^{-2}$ in the blue, red, and far-red regions, respectively (measured by International Light Co. 150 Photometer), were achieved by using a 3·2:1 input wattage ratio of cool-white fluorescent to incandescent bulbs ($22{\cdot}5 \times 10^3$ Lux). There was no barrier between lights and growing area and walls of the chamber were covered with aluminized Mylar. Chambers were located in a room supplied with air conditioned to 22°C and filtered by passage through roughing and bag filters, a packed-tower scrubber, and, finally, activated charcoal filters. Air from the room passed through additional roughing, bag, and charcoal filters before entering the chambers from the bottom and moving upward past the plants. Air was not recirculated and flow rates in the chambers were approximately 0·21 m/sec.

Generation and monitoring of gases

SO_2 was introduced by metering the gas from a cylinder containing 20% v/v SO_2 in nitrogen gas through a pressure regulator equipped with a micrometer needle valve. O_3 was generated using an Orec Ozonator and a metered supply of oxygen. The concentration of SO_2 in the chamber was measured continuously with a conductometric analyzer (Scientific Industries) calibrated for SO_2 concentration with standard SO_2 permeation tubes.[7] O_3 concentrations were measured with a coulometric analyzer (Mast Ozone Meter) calibrated for air and liquid flow rates and for O_3 concentration using an O_3 source and parallel colorimetric KI measurements.[7] A chromic acid scrubber on the Mast meter eliminated interference by SO_2. Teflon tubing was used to sample air from the center of the chambers at a height level with the tops of the plants.

During the course of the experiments, ambient CO_2 concentrations were monitored intermittently in the chambers with a Beckman Infrared Analyzer calibrated with CO_2 at 300 and 600 μl/l. The range of CO_2 concentrations found

was from 305 to 465 μl/l (normal concentrations at sea level are about 316 μl/l). Elevated CO_2 concentrations in the chambers occurred on days of poor local atmospheric dispersion conditions and were accompanied by the presence of NO_2 (less than 0·1 μl/l). Although elevated concentrations of these gases could have an effect on stomatal function,[19] these occurrences were infrequent, occurred in all chambers, and the effects of treatments performed on these days did not appear to be different from effects obtained on other days.

Symptom evaluation

After the period of exposure, Tempo bean plants were returned to a greenhouse receiving filtered-air for 2 to 4 days to permit symptoms to develop. Unifoliate leaves of bean and mature leaves of tobacco were scored for the presence or absence of injury and the percent of leaves injured was used as a measure of the incidence of foliar injury. The relative amount of leaf area injured also was estimated by rating each leaf on a scale of 0 (no injury) to 5 (100% of leaf area injured) for bean and 0 (no injury) to 10 (100% of leaf area injured) for tobacco. These injury scores were used as estimates of the severity of foliar injury.

Experimental design and statistical analysis

In the first series of tests, plants were exposed to each of 5 concentrations of O_3 (0·1, 0·2, 0·3, 0·4, or 0·8 μl/l) in the presence and in the absence of 0·4 μl/l SO_2. Treatments were performed in random sequence until 2 exposures of each SO_2–O_3 combination were completed. In the 2nd series of tests, plants were exposed to each of 5 concentrations of SO_2 (0·4, 0·6, 0·8, 1·2, 1·6 μl/l) in the presence and absence of 0·07 μl/l O_3. Fumigations were first performed in stepwise sequence beginning with 0·4 μl/l, then replicate fumigations were carried out in random order to provide 2 exposures at each concentration of the individual and combined pollutants. Later, exposures were performed to obtain additional information at concentrations of 0·07 and 0·15 μl/l O_3 and 0·5 μl/l SO_2. All exposures were for a duration of 4 hr.

The number of individual leaves scored for each treatment was 40 to 160 for Tempo bean, 24 to 64 for Red Kidney bean, and 26 to 151 for tobacco. Chi-square analysis with continuity correction[10] was used to test the null hypothesis that there was no difference between the incidence of foliar injury in the presence and in the absence of the constant pollutant. Fisher's exact probability test[10] was used with less than 40 individual leaves in a treatment. Dose–response curves were constructed for all cultivars by plotting incidence of foliar injury against concentration. The data on incidence of leaf injury in Tempo bean then were transformed to probit units following the procedures of FINNEY[8] and FISHER and YATES[9] and plotted against the logarithm of pollutant concentration. Regression analyses were performed on these functions and the median effective doses (ED_{50} values) were calculated from the probit regression curves (Tempo bean) or the dose–response curves (Red Kidney bean and tobacco) both for the individual and combined pollutants.

RESULTS

Reactivity of SO_2 and O_3 in the chamber atmosphere

In preliminary tests, measurements of SO_2 concentration in a chamber were made before and during addition of O_3. Likewise, measurements of O_3 were performed before and during addition of SO_2 to the chamber. At concentrations in the same range as those used in the experimental fumigations, neither SO_2 nor O_3 concentrations changed when the other pollutant was introduced. Thus, there was no evidence that reactions occurred between SO_2 and O_3 which would lower the gas phase concentrations of either gas.

Symptom expression

Symptoms of SO_2 and O_3 injury on bean leaves consisted of tan to white interveinal necrotic lesions. Lesions resulting from exposure to SO_2 were more sharply defined than those of O_3 and, with time, the discolored zones coalesced into large, irregularly-shaped areas. Both abaxial and adaxial portions of leaves were affected. Injury symptoms on tobacco leaves were similar to those of bean but they were not confined to interveinal portions exclusively. Symptoms on plants exposed to the combined pollutants were similar to either SO_2 or O_3 symptoms depending on which pollutant was at a concentration above its threshold for injury.

Comparative phytotoxicity of SO_2 and O_3

O_3 was considerably more injurious than SO_2 to

Tempo bean (Table 1), Red Kidney bean (Table 2), and tobacco (Table 3) on a v/v concentration basis. The lowest concentrations of individual pollutants at which foliar injury occurred in these tests were: 0·1 μl/l O_3 and 0·4 μl/l SO_2 to leaves of Red Kidney bean, 0·2 μl/l O_3 and 0·6 μl/l SO_2 to leaves of Tempo bean, and 0·2 μl/l O_3 and 0·6 μl/l SO_2 to leaves of Bel-W 3 tobacco. The incidence of foliar injury increased in proportion to the concentration of pollutants supplied rising from 0 to 100% of leaves injured for an increase in O_3 concentration of from 0·2 to 0·8 μl/l in Tempo bean, 0·2 to 0·8 μl/l in tobacco, and 0·07 to 0·3 μl/l in Red Kidney bean or from 0·6 to 1·6 μl/l SO_2 in Tempo bean and 0·4 to 0·8 μl/l SO_2 in Red Kidney bean. Tobacco was tolerant of SO_2 and concentrations employed were not sufficiently high to cause injury to 100% of tobacco leaves. The severity of lesions (foliar injury scores) also increased in proportion to the concentration of pollutants supplied but injury scores varied more widely than incidence of foliar injury from one replicate test to another.

Effect of SO_2 at different O_3 concentrations

In the presence of 0·4 μl/l SO_2, the incidence of

Table 1. *Effect of exposure to SO_2 and O_3 on injury to unifoliate leaves of* 9*-day-old* P. vulgaris *cv. Tempo*

Concn. (μl/l) SO_2	O_3	Total no. of leaves	No. leaves injured	Mean injury score*	Statistical significance†
0·4	0	80	0	0	
0	0·1	80	0	0	
0·4	0·1	160	0	0	
0	0·15	40	0	0	
0·4	0·15	40	0	0	
0	0·2	160	45	0·5	N.S.
0·4	0·2	160	54	0·2	
0	0·3	64	16	0·3	N.S.
0·4	0·3	64	13	0·13	
0	0·4	168	156	1·3	0·01
0·4	0·4	120	85	0·8	
0	0·8	80	80	2·7	N.S.
0·4	0·8	130	129	2·4	
0	0·07	80	0	0	
0·4	0	80	0	0	
0·4	0·07	80	0	0	
0·5	0	48	0	0	
0·5	0·07	48	0	0	
0·6	0	80	26	0·2	0·01
0·6	0·07	80	3	0·02	
0·8	0	80	50	0·5	0·01
0·8	0·07	80	21	0·1	
1·2	0	80	78	2·8	0·01
1·2	0·07	80	59	0·4	
1·6	0	80	80	4·1	N.S.
1·6	0·07	80	76	1·7	

* Injury scores ranged from 0 (no injury) to 5 (100% of leaf area injured).

† Null hypothesis tested: no difference between incidence of leaf injury with and without second pollutant. N.S. = null hypothesis not contradicted. 0·01 = null hypothesis contradicted at $p \leqq 0{\cdot}01$.

Table 2. Effect of exposure to SO_2 and O_3 on injury to unifoliate leaves of 14-day-old P. vulgaris *cv. Red Kidney*

Concn. μl/l SO_2	Concn. μl/l O_3	Total no. of leaves	No. leaves injured	Mean injury score*	Statistical significance†
0·4	0	64	8	0·2	
0	0·1	48	31	0·39	0·01
0·4	0·1	48	6	0·06	
0	0·2	64	44	1·3	0·01
0·4	0·2	64	4	0·05	
0	0·3	40	40	2·2	N.S.
0·4	0·3	40	40	2·0	
0	0·4	68	68	3·1	N.S.
0·4	0·4	48	46	1·8	
0	0·8	32	32	5·0	N.S.
0·4	0·8	32	32	4·5	
0	0·07	32	0	0	
0·2	0	16	0	0	
0·2	0·07	16	0	0	
0·4	0	64	8	0·2	0·05
0·4	0·07	32	0	0	
0·5	0	16	0	0	
0·5	0·07	16	0	0	
0·6	0	32	29	2·3	0·05
0·6	0·07	32	25	0·6	
0·8	0	32	32	2·4	0·05
0·8	0·07	32	24	0·5	
1·2	0	32	32	4·8	0·05
1·2	0·07	32	28	3·1	
1·6	0	32	32	5·0	N.S.
1·6	0·07	32	32	4·8	

* Injury scores ranged from 0 (no injury) to 5 (100% of leaf surface injured).

† Null hypothesis tested: no difference between incidence of leaf injury with and without second pollutant. N.S. = null hypothesis not contradicted. 0·01, 0·05 = null hypothesis contradicted at $p \leqq 0{\cdot}01$ or $p \leqq 0{\cdot}05$ respectively.

foliar injury caused by different concentrations of O_3 was significantly reduced in leaves of Tempo bean (Table 1) and Red Kidney bean (Table 2). However, the incidence of injury to foliage of tobacco caused by 0·1 μl/l O_3 was significantly increased in the presence of 0·4 μl/l SO_2 (Table 2). Injury scores provided corroborative evidence that SO_2 attenuated the effects of O_3 in bean and enhanced the effects of O_3 in tobacco.

Effect of O_3 at different SO_2 concentrations

In the presence of 0·07 μl/l O_3, the incidence of foliar injury caused by different concentrations of SO_2 was significantly reduced in both Tempo bean (Table 1) and Red Kidney bean (Table 2). However, the incidence of injury to foliage of tobacco caused by 1·6 μl/l SO_2 was significantly increased in the simultaneous presence of 0·07 μl/l O_3 (Table 3). Injury scores also confirmed that O_3 attenuated the effects of SO_2 in bean and promoted the effects of SO_2 in tobacco.

Analysis of dose–response curves

Response (precentage of leaves injured) was approximately a logarithmic function of dose (concentration of pollutant applied over a 4-hr

Table 3. Effect of exposure to SO_2 and O_3 on injury to leaves of N. tabacum *cv. Bel-W 3*

Concn. (μl/l) SO_2	Concn. (μl/l) O_3	Total no. of leaves	No. leaves injured	Mean injury score*	Statistical significance†
0·4	0	27	0	0	
0	0·1	39	0	0	0·01
0·4	0·1	62	18	0·6	
0	0·2	51	39	1·6	N.S.
0·4	0·2	41	30	0·9	
0	0·4	61	59	3·1	N.S.
0·4	0·4	56	56	3·9	
0	0·8	44	44	5·9	N.S.
0·4	0·8	28	28	3·8	
0	0·07	25	0	0	
0·4	0	38	0	0	
0·4	0·07	39	0	0	
0·6	0	26	1	0·06	N.S.
0·6	0·07	28	1	0·08	
0·8	0	41	0	0	
0·8	0·07	44	0	0	
1·2	0	40	10	0·2	N.S.
1·2	0·07	30	7	0·2	
1·6	0	144	49	0·6	0·01
1·6	0·07	151	100	2·0	

*Severity of injury ranged from score of 0 (no injury) to 10 (100% of leaf area injured).

†Null hypothesis tested: no difference between incidence of leaf injury with and without second pollutant. N.S. = null hypothesis not contradicted. 0·01 = null hypothesis contradicted at $p \leqq 0{\cdot}01$.

period) when the data for incidence of injury to Tempo bean leaves was converted into probit units (Fig. 1). Correlation coefficients for these functions were greater than 0·9 in all cases and were statistically significant at $p \leqq 0{\cdot}1$. The slopes of the probit regression curves for Tempo bean leaves exposed to mixtures of SO_2 and O_3 were lower than for the individual pollutants, but the differences in slopes were not statistically significant. Similar probit regression curves were constructed for Red Kidney bean and tobacco (Fig. 1) but the more variable response of plants grown in the greenhouse resulted in regressions which were not significant at $p \leqq 0{\cdot}1$.

ED_{50} values (median effective doses) for incidence and severity of foliar injury by the individual and combined pollutants were estimated in order to indicate the magnitude of interactive effects (Table 4). SO_2 at 0·4 μl/l raised the ED_{50} for incidence of O_3 injury to bean leaves

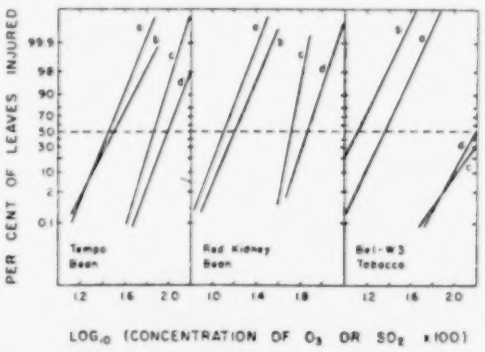

FIG. 1. Probit regression lines showing effect of ozone on the toxicity of sulfur dioxide and the effect of sulfur dioxide on the toxicity of ozone in *P. vulgaris* cv. Tempo and Red Kidney and *N. tabacum* cv. Bel-W3. a: O_3; b: O_3 with 0·4 μl/l SO_2; c: SO_2; d: SO_2 with 0·07 μl/l O_3.

Table 4. Median effective doses (ED_{50}) for the incidence and severity of foliar injury in bean and tobacco after exposure to O_3, SO_2, and mixtures of O_3 and SO_2

	Concentration producing injury on:							
	50% of leaves*				50% of leaf area†			
	O_3	O_3 with SO_2‡	SO_2	SO_2 with O_3§	O_3	O_3 with SO_2‡	SO_2	SO_2 with O_3§
	(μl/l)							
P. vulgaris cv. Tempo	0·31	0·36	0·79	1·0	0·74	0·85	0·9	1·3
P. vulgaris cv. Red Kidney	0·14	0·17	0·54	0·86	0·33	0·46	0·82	1·08
N. tabacum cv. Bel-W 3	0·23	0·13	2·0	1·6	ND	ND	ND	ND

* ED_{50} values estimated from probit regression curves.
† ED_{50} values estimated assuming linear relationship between mean injury score and concentrations of pollutants.
‡ Constant concentration of SO_2: 0·4 μl/l.
§ Constant concentration of O_3: 0·07 μl/l. ND = no data available.

by 6 to 21%. O_3 at 0·07 μl/l raised the ED_{50} for incidence of SO_2 injury to bean by 25 to 45% and lowered the ED_{50} for tobacco by about 25%. Higher concentrations of pollutants were required to injure 50% of the leaf area than to cause injury to 50% of the leaves and the ED_{50} values for severity of injury (foliar injury scores) also were altered in the presence of mixtures of SO_2 and O_3 (Table 4).

DISCUSSION AND CONCLUSIONS

Our results indicate that dose–response relationships for frequency of foliar injury caused by SO_2 and O_3, individually and in combination, approximate binomial functions similar to the effects of many other toxic agents on biological organisms. A previous report indicated that foliar injury to tobacco and bean plants caused by exposure to O_3 produced a sigmoidal dose–response function.[12] MacDowall and Cole[17] also found the probit conversion of value in assessing interactive effects of O_3 and SO_2 on tobacco.

There are 4 possible types of interactive effects demonstrated by this study. In one test with tobacco, a mixture of concentrations of SO_2 and O_3 produced foliar injury even though each pollutant did not when applied alone. Conversely, in one test with bean plants, a mixture of concentrations of SO_2 and O_3 produced no foliar injury even though the individual pollutants were toxic to foliage. In several tests, injury was enhanced in tobacco or suppressed in bean in the combined presence of concentrations of SO_2 and O_3 which individually were injurious.

We have concluded that the incidence of injury to foliage of bean and tobacco produced by combinations of SO_2 and O_3 cannot be estimated solely from knowledge of the effects of the individual pollutants. The occurrence of enhanced or attenuated effects in the presence of certain combinations of SO_2 and O_3 depended on the plant species: with bean plants, incidence of injury was attenuated, but with tobacco, injury was enhanced. The occurrence of these interactive effects also depended on the concentrations of pollutants: at the higher concentrations of SO_2 or O_3 all leaves were injured, consequently, no change in incidence of injury by mixtures of pollutants could be demonstrated. These results are in agreement with some previous reports concerning interactive effects of air

pollutants.[25–27] However, it apparently is not widely known that concentrations of SO_2 or O_3 which do not exceed the respective U.S. ambient air quality standards can alter the incidence of injury caused by the other pollutant.

Dose–response relationships describing the effects of a toxic agent on plants are particularly useful because they allow interpolation between measuring points, can be used to indicate the threshold for injury, and provide a firm basis for clarifying the combined effects of toxic agents. In this study, these relationships were established for SO_2 and O_3, alone and in combination, by transforming incidence of foliar injury into probit units and presenting the data as logarithmic functions of dose (concentration applied over a 4-hr period). Although regressions of the individual functions were significant, the variation in foliar response with pollutant concentration and the narrow range of concentrations over which incidence of injury increased from 0 to 100% did not provide data of sufficient precision for comparisons of the slopes and intercepts of the dose–response curves. However, with certain combinations of SO_2 and O_3, the combined effects were significantly different from the effects of single pollutants. In a previous study with tobacco, MacDowall and Cole[17] found that the slopes of regression lines were more than additive for mixtures of SO_2 and O_3 and the threshold for injury by SO_2 was lowered but not the thresnold for injury by O_3.

There are two important applications for these findings, one relates to ambient air quality standards and the other to future efforts to demonstrate enhancement or attenuation of effects on vegetation by mixtures of pollutants.

When air quality standards were originally established,[7] there was little information available on interactive effects. Now that more data are available, interactive effects can be considered in future revisions of these standards, however, it is not possible to apply a constant principle for all plant species. Variation in the direction and magnitude of interactive effects with species and with pollutant concentrations indicates that no modification of current SO_2 or O_3 secondary air quality standards can be recommended at the present time. Further investigation of the extent and ubiquity of interactive effects is needed and specific attention should be directed toward the study of concentrations of SO_2 or O_3 which do not violate current standards. Experiments to determine effects on growth and yield should be performed[18,26] to provide systematic evaluations of dose–response relationships.[13,17]

There have been few, if any, attempts to explain interactive effects, yet several possible mechanisms for these effects exist. For example, pollutants may react chemically with each other before contact with foliage, at the leaf surface, or after entry into leaves. The effective concentrations of these pollutants then would decrease and the reaction products may be more or less injurious than the original substances. On the other hand, one pollutant may inhibit or promote foliar uptake of the other pollutant by altering stomatal resistance to diffusion of gases.[19] Finally, one pollutant may cause a change in physiological functions or in metabolic reactions which alters the susceptibility of the plant to the other pollutant.[30] Costonis[6] obtained a reduction in SO_2 injury by O_3 in Eastern white pine but this appeared to result from reactions between SO_2 and O_3 on surfaces of devices used to control pollutant flow rates after these pollutants were monitored but before they contacted vegetation. We investigated this possibility in our experiments and found no evidence of a change in either SO_2 or O_3 concentrations on introduction of the other pollutant. Other investigators also have found no reactions in the gas phase between SO_2 and O_3 at low concentrations.[14,22] Consequently, we believe that explanations for the interactive effects found in our study lie either in effects on stomatal function or in altered physiologic or metabolic processes.

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ADDENDUM

The following pertinent articles appeared after the manuscript was written. Larsen R. I. and Heck W. W. (1976) An air quality data analysis system for interrelating effects, standards, and needed source reductions: Part 3. Vegetation injury. *J. Air Pollut. Control Assoc.* **26,** 325–333. Reinert R. A., Heagle A. S. and Heck W. W. (1975) Plant responses to pollutant combinations. Pages 159–178 *in* J. B. Mudd and T. T. Kozlowski, eds. *Responses of plants to air pollution*, Academic Press, New York.